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THE ROLE OF MITOCHONDRIA IN THE DETECTION OF INFRARED LIGHT SOURCES BY MAMMALIAN CELLS.

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14. ABSTRACT
1. We have discovered a novel expression of the function of live mitochondria, which we called '**R**eversible **E**xcitation **L**ight **I**nduced **E**nhancement of **F**luorescence (**RELIEF**)' It appears restricted to mitochondria of mammalian cells. Virus infected cultured cells change their expression of RELIEF within hours of infection. Neurons in culture express RELIEF quite strongly and change the expression as they develop, age and degenerate.It offers a simple fluorescence assay that can be completed in minutes.It can be observed in live cells, in the extracellular medium, and in isolated mitochondria.
2. The secretion of the fluorescent material into the extracellular medium led us to a search for the secretory channels. We found a class of new objects which we call 'I-centers' (water structuring centers) on the cell surface. At present it is not yet clear whether they related to the secretion channels of the RELIEF-related materials.

15. SUBJECT TERMS

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LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED:

1. Albrecht-Buehler, G. Reversible excitation light-induced enhancement of fluorescence of live mammalian mitochondria. (2000), FASEBJ. 14: 1864- 1866 (full paper at http:// www.fasebj.org/ cgi/ reprint/ 00-0028fjev1
2. Albrecht-Buehler, G. Water structuring centers of mammalian cell surfaces. (2002) Experimental Cell Res. (in press)

SCIENTIFIC PERSONNEL SUPPORTED BY THIS GRANT

Guenter Albrecht-Buehler, Ph.D., P.I.

BRIEF OUTLINE OF RESEARCH FINDINGS

1. The excitation-induced enhancement of fluorescence (RELIEF)

Using a self-designed microspectrograph the crucial finding of the project is the observation that one or several peaks of the autofluorescence spectrum of the mitochondria of live mammalian cells flared up 3fold or more if the cells were irradiated for 2-3 minutes with ultraviolet light (λ= 365 nm) (Fig. 1)

Subsequently the peaks remained stable at this new level of intensity for 30 min or longer. Within the limits of resolution of ±10 nm of the micro-spectrograph the location of the emission peaks did not change.

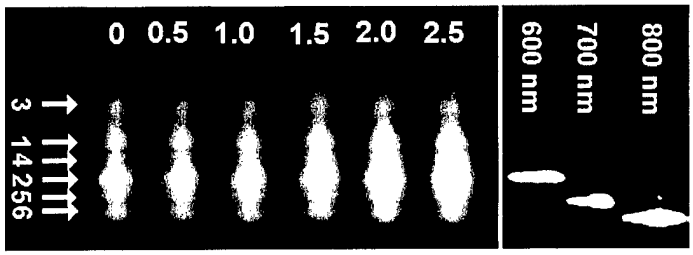

Fig. 1: Fluorescence enhancement of 3T3 cells at 37° C on a cover slip irradiated with near-ultraviolet light (λ= 365±10 nm; 16 $\mu W/mm^2$). The numbers at the top of each panel indicate the duration of irradiation in minutes. The 3 panels on the right are the spectra of narrow band interference filters (peaks of transmission indicated on the panels) recorded under identical conditions as the cells. The numbers on the left-hand panel indicate the 2 major peaks emitted (λ_1= 530 nm and λ_2=600 nm).

The effect was reversible, i.e. (a) the induced increase of fluorescence could be reversed by turning off the excitation light for a certain period of time, and (b) the effect could be re-stimulated using the same cells. Therefore, the effect was not due to UV damage of the cells.

In addition to 3T3 and CV1 cells we found that cells from other placental mammals such as HeLa cells and BHK cells expressed RELIEF, as well. Not only cultured cells, but also cells isolated directly from tissue expressed RELIEF. For example, rat liver homogenates expressed RELIEF very strongly.

When we tested cells from organisms other than placental mammals, we did not find expression of RELIEF. The tested cell types included avian cells (chick embryo fibroblasts SL-29), amphibian cells (homogenates

of Xenopus laevis liver), insect cells (Drosophila melanogaster cell line Kc167), nematode cells (intact worms and homogenates of Caenorhabditis elegans), yeast cells (Saccharomyces cerevisiae), and slime mold cells (Dictyostelium discoideum). In addition, PtK1 cells (Potorous tridactylis) were tested as an example of marsupial mammalian cells. They did not express RELIEF, either.

RELIEF was found to be insensitve to drugs that inhibit protein synthesis or cytoskeletal elements. In contrast, it was inhibited by drugs that inhibited mitochondrial function, such as KCN , Na-azide, or 2.7 mM 2,4-dinitro-phenol.

Consistent with this interpretation that RELIEF originated in the mitochondria, cells or cell fractions without mitochondria such as RBCs (the author's own) or isolated nuclei of CV1 cells (isolated by the procedure described in (11) did not express RELIEF. On the other hand, destructive cell treatments that left mitochondria or at least their inner membrane intact continued to support RELIEF. For example, cell homogenates generated by intense douncing of rat liver expressed RELIEF very intensely. Likewise, incubation of CV1 cells with 1% of the non-ionic detergent NP40 did not inhibit RELIEF, although the time required to reach the active level was increased 4-fold to 24 min.

Preliminary studies showed that preparations of mitochondria from mouse liver did not express RELIEF. Instead, the fluorescence faded. However after suspending the mitochondria in normal culture medium to which 10 mM Na-pyruvate was added, the fluorescence recovered within 4 minutes and, subsequently, expressed RELIEF.

Quantitiation of RELIEF

We developed a quantitiation of RELIEF that tests

a. the rise of autofluorescence

b. the subsequent decay of autofluorescence

c. the recovery of autofluorescence.

Figure 2. shows a typical measurement which selects a small area in the autofluorescent image, called a 'probe', and computes the average pixelvalue inside this probe. The amplitude of RELIEF, its rate of rise and decay and the amplitude of recovery are measured from the time course of the probe-data.

Virus infected cultured cells change their expression of RELIEF within hours of infection (Collaboration with Prof. Kathy Rundell, Northwestern Uninversity medical School).

Using this assay, we tested whether virus infection would alter the expression of RELIEF. Using the infection of CV1 cells with SV40 virus as a test system, we found, indeed, drastic changes in the expression of RELIEF upon the pregression of the infection long before morphological alterations could be observed. Fig. 3 shows an example. Within one day after the infection by SV40 viruses, the expression of RELIEF dropped by almost 30%.

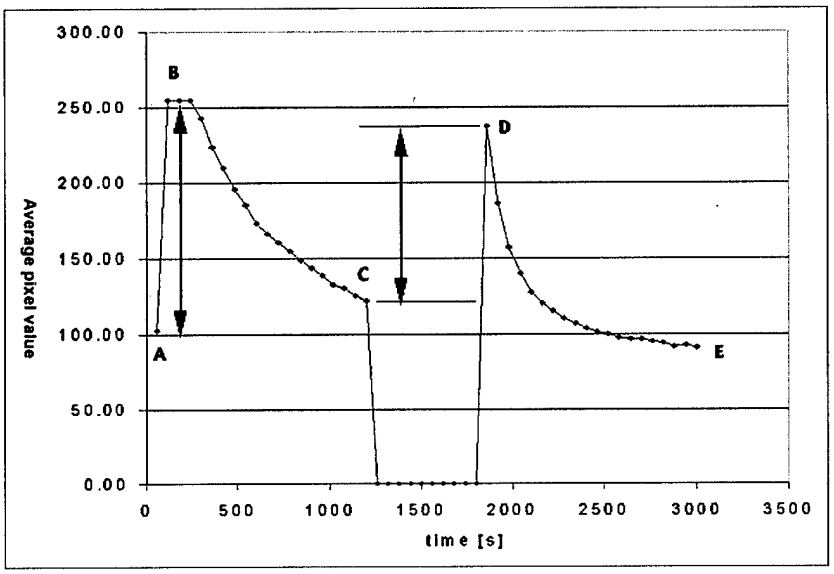

Figure 2. Quantitation of RELIEF. Amplitude A_R of RELIEF = (level B - level A)/level A; Decay rate D_1 = exponent of segment B to C obtained by curve fitting; Recovery of RELIEF = (level D - level C)/level C; Decay rate D_2 = exponent of segment D to E obtained by curve fitting.

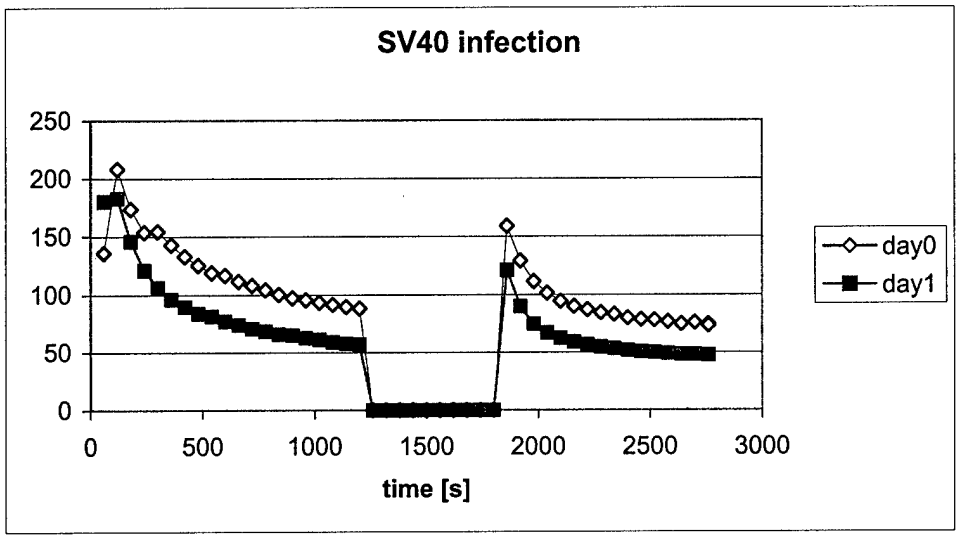

Figure 3. Expression of virus infection on the expression of RELIEF BY CV1 cells .

<u>Neurons in culture express RELIEF quite strongly and change the expression as they develop, age and degenerate. (In Collaboration with Prof. Adriana Ferreira, Northwestern University Medical School)</u>

Using cultured rat pyramidal cells as a model system of neural aging and degeneration, we found that the expression of RELIEF changed markedly as the cells developed and aged in culture. (Fig. 4)

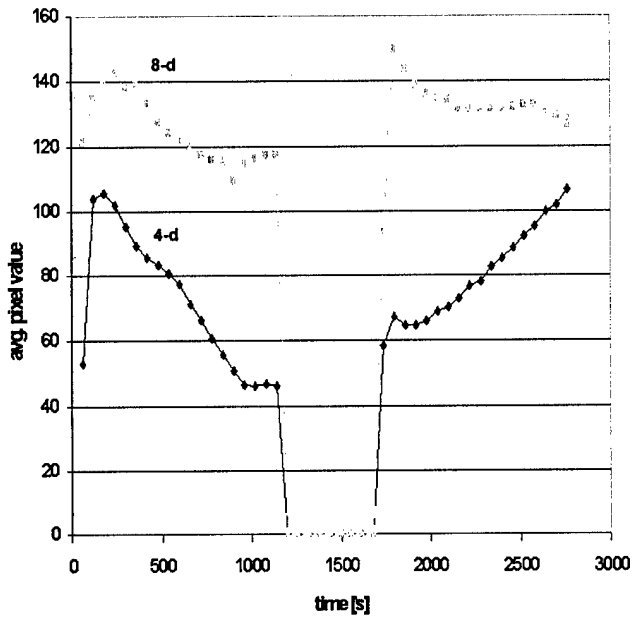

Figure 4. Expression of RELIEF in naturally aging rat pyramidal cell cultures. The experimental protocol consisted of 20 min exposure to 365 nm light, a 10 min recovery dark period and another 20 min exposure of the identical cells with 365 nm light. The lower curve marked '4-d' indicates the response of 4-day old cultures; the upper curve ('8-d') indicates the response of 8-day old cultures.

RELIEF can be observed not only in live cells, and in isolated mitochondria, but also in the extracellular medium.

The above findings suggested that the expression of RELIEF may be a very useful and novel assay to study the progression of dieseases that are caused by infection or neurodegeneration, or other pathologenic agents and processes. Therefore, it would be very important to isolate the RELIEF - specific, autofluorescent material. At this point it seemed that one would have to purify the material from isolated mitochondria. Such a method appeared quite difficult, because the starting material is heavily contaminated with cellular materials. We were fortunate, however, to find that one can begin the purification with very pure and simple starting materials, namely serum-free extracellular salt solutions. The finding is shown in Figure 5 which demonstrates that the autofluorescence can be rapidly removed by streaming fresh medium to the cells. Subsequently, the autofluorescence is restored by the cells. The result demonstrates that some of the RELIEF-specific material is secreted to the extracellular medium, from where it can be harvested for further purification. Since the extracellular medium can be chosen to be a simple buffer solution, the starting material for the purification can be chosen to be chemically simple and little contaminated with any other organic material.

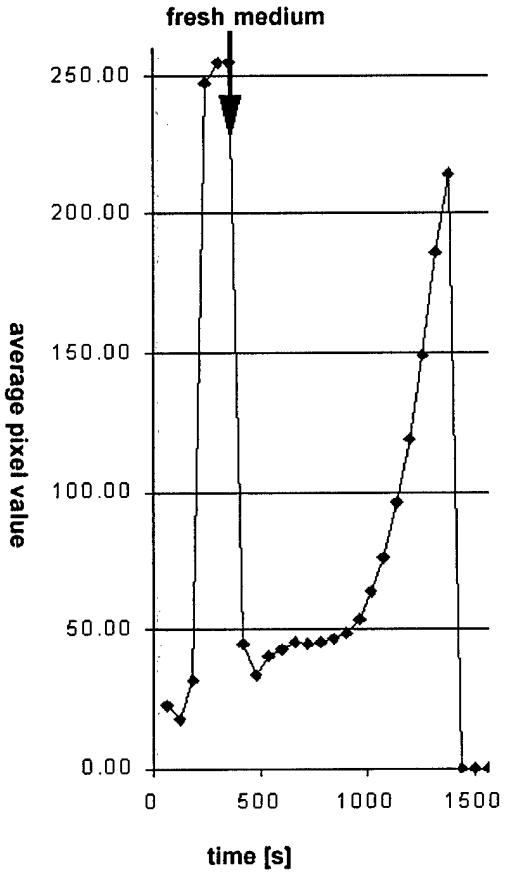

Figure 5. Evidence for the extracellular localization of RELIEF-specific fluorescent material (Probe is located in the space between cells.

2. Water structuring centers of mammalian cell surfaces

Cultured mammalian cells appeared to express specific particles on their surface, which could be detected by their ability to nucleate ice crystals (I-centers) in a newly developed, 2-dimensional crystallization assay (Figure 6). Their expression required approximately 24h independent of cell density, required metabolic energy, and the number and distribution of the I-centers were cell-type specific. Their characteristic ability to nucleate ice crystals was highly sensitive to dehydration, to hyaluronidase and phospholipase C, but not to a number of proteases such as trypsin, chymotrypsin, collagenase and pronase. However, these proteases, especially pronase, were able to detach the I-centers from the cell surface, without destroying their ability to nucleate ice crystals. I-centers were specific products of live cells, located in relatively small numbers at the cell surface organized in a detachable, sheet-like structure.

We propose to consider the ice nucleating ability of I-centers as an expression of their ability to influence the water structure in the surface of cells. And even though their biological function is not known at this time, as water-structuring centers they appear remarkable enough to warrant our attention.

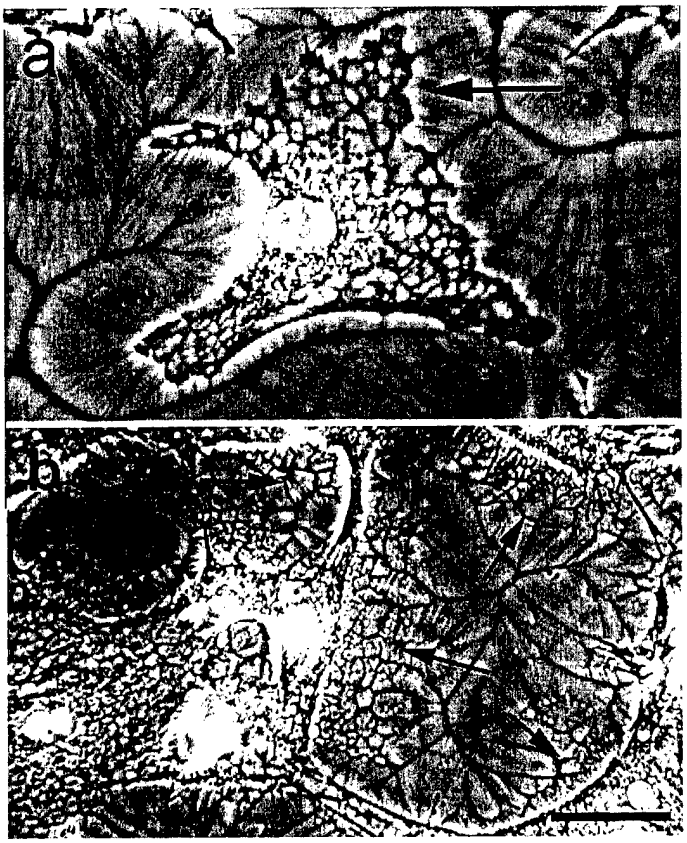

Figure 6. Phase dense structures are the outlines of ice crystals (called 'coronas') that are nucleated by I-centers and depicted by our newly developed method. The panels show dorsal honeycomb-like coronas and intercellular cell coronas (Bar indicates 50 μm). a. Typical appearance of honeycomb coronas. Honeycomb coronas appearing on top of thin peripheral parts of cell bodies (arrow). b. Sheet-like organisations of honeycomb coronas. After growing for 1 day on coverslips 3T3 cells were placed for 4 hours on ice. As they retracted without rounding up, they left the areas of honeycomb coronas behind and appeared continuous with the extracellular coronas (arrows).

HONORS

1. An article ("How do cells see each other" by Colin Lowry) about the P.I.'s past work in "21st CENTURY Science & Technology" :p. 74-77(Spring 2001).
2. An article ("Body talk" by Bennett Daviss) about novel photobiological findings including a chapter about the P.I.'s work in "NewScientist (23 February 2002 # 2331)

INTERACTIONS/TRANSITIONS

a. Participation at meetings where the work was presented

2/2-3/99	Speaker, MURI Research meeting (AFOSR)
4/22-23/99	Keynote speaker; retreat of the LCMB of the NIH, Harpers Ferry
2/9/2000	Department of Biology, University of Cincinnati
3/7/2000	Department of Biology, University of Illinois at Chicago
4/20/2000	Department of Neurobiology, Baylor College of Medicine
4/25/2000	Department of Electrical Engineering , University of Colorado, Boulder
1/16-1/30/02	37.the Winter Seminar, Klosters, Switzerland

b. Transitions

RELIEF may offers a much needed, rapid test to determine whether soldiers or civilians have been exposed to chemical or biological weapons. As mentioned above, there is an effect of virus infection on the expression of RELIEF in mammalian cells. As a practical application one may consider to develop a handheld, combat-ready device consisting of a 365 nm light source and an imaging system to measure RELIEF (i.e. the change of autofluorescence of mitochondria) in the skin of soldiers, within hours of a suspected exposure to these weapons. Since in most cases early detection allows the application of effective counter-measures, the device could offer an effective defense.